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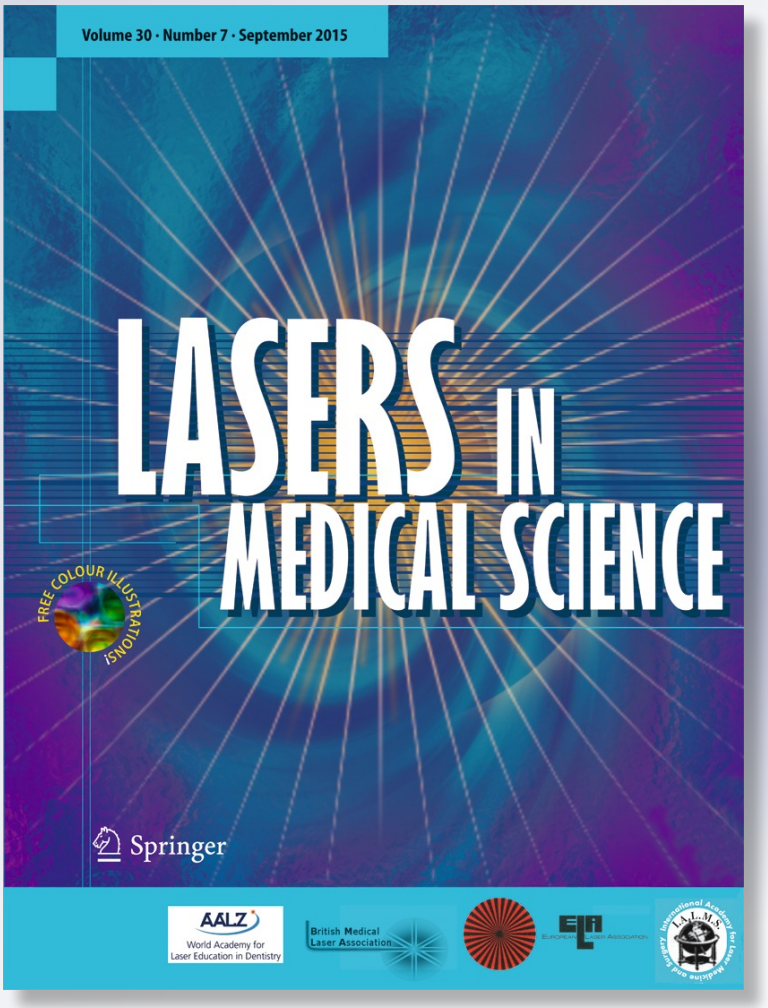

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ORIGINAL ARTICLE

Intense pulsed light induces synthesis of dermal extracellular proteins in vitro

E. Cuerda-Galindo[1] · **G. Díaz-Gil**[1] · **M. A. Palomar-Gallego**[1] · **R. Linares-GarcíaValdecasas**[1]

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Abstract Intense pulsed light (IPL) devices have been shown to be highly effective for the skin rejuvenation. In our study, we try to elucidate effects of IPL in fibroblast proliferation, in gene expression, and in extracellular matrix protein production. 1BR3G human skin fibroblasts were used to test the effects of an IPL device (MiniSilk FT, Deka®). Fibroblasts were divided into three groups: group 1 was irradiated with filter 800–1200 nm (frequency 10 Hz, 15 s, fluence 60.1 J/cm) twice; group 2 was irradiated with filter 550–1200 nm (double pulse 5 ms+5 ms, delay 10 ms, fluence 13 J/cm2) twice; and group 3 was irradiated with filter 550–1200 nm (frequency 10 Hz, 15 s, fluence 60.1 J/cm2) twice. To determine changes in gene expression, messenger RNA (mRNA) levels for collagen types I and III and metalloproteinase 1 (MMP-1) were performed 48 h after irradiation. To determine changes in hyaluronic acid, versican, and decorin, mRNA and ELISA tests were performed after 48 h of treatment. In addition to this, a Picro-Sirius red staining for collagen was made. The study showed an increase of mRNA and hyaluronic acid, decorin, and versican production. With RT-PCR assays, an increase mRNA for collagen type I, type III, and MMP-1 was observed. Collagen and hyaluronic synthesis was increased in all groups with no differences among them, while decorin and versican synthesis was higher in those groups irradiated with 550–1200-nm filters with no dependence of type pulse or total energy dose. IPL applied in vitro cultured cells increases fibroblasts activity. Synthesis of extracellular proteins seems to be produced more specifically in determined wavelengths, which could demonstrate a biochemical mechanism light depending.

Keywords Collagen · Decorine · Fibroblast · Hyaluronic acid · Versican

✉ E. Cuerda-Galindo
esther.cuerda@urjc.es

[1] Section of Human Anatomy and Embryology, Universidad Rey Juan Carlos, Avda Atenas sn, 28922 Alcorcón, Madrid, Spain

Introduction

The human skin aged includes histological and biochemical changes such as thinning epidermis. In dermis, collagen fibers appear thickened and fragmented with higher ratio of collagen III to collagen I [1], with loose, straight, and decreased fibers [2]. Elastic fibers become structurally and functionally abnormal, with deposit of abnormal elastic fibers, lower degradation, and gradual accumulation of solar elastotic material in the upper dermis [3]. Changes in polysaccharide and proteoglycans of extracellular matrix are reported with abnormal localization and structure [4]. Because of the changes in dermal ground substance, efficient dermal hydration cannot be properly maintained [5] and all these changes manifest clinically as dry and fragile skin.

Intense pulsed light (IPL) devices use flashlamps and bandpass filters to emit polychromatic incoherent high-intensity pulsed light of determined wavelength spectrum, fluence, and pulse duration [6]. IPL sources are multiwavelength light that typically emit light in the 500- to 1200-nm range. In order to achieve target selectivity, various cutoff filters are employed, effectively removing lower wavelengths. The therapeutic technology of noninvasive skin rejuvenation of IPL is called photorejuvenation, and the technique has been used widely in cosmetic dermatology to improve facial photoaging [7]. The efficacy of IPL in remodeling the extracellular matrix of aged skin had been proven by an increasing number of clinical trials. Some author argue that, although

the exact mechanism of photorejuvenation following IPL treatments is not completely clear, dermal heating likely results from some absorption by water, as well as propagation of heat from the superficial vasculature [8]. Stimulation of fibroblast and subsequent neocollagenesis and dermal remodeling, increased epidermal thickness, decreased horny plugs, formation of new rete ridges, and decreased elastosis have been noted histologically after 6 months following treatment and can contribute to clinical improvement [9]. However, its molecular biological mechanism and signaling pathway for treatment is rarely reported.

For treating patients with aging skin, high cutoff filters are used to obtain wavelengths higher than 800 nm (near infrared) to obtain collagen denaturation by heating. Thermal denaturation take place at 63 to 64 °C and can occur over a range of temperatures and pulse durations with observable changes in staining and structural collagen fibers [8]. There are clinical reports of improvement and some histological studies using different wavelengths [10, 11] although some authors argue that collagen denaturation is insufficient as a sole and direct corollary for effective treatment [12]. To date, scarce data are reported on the effects of IPL on human skin cells and the associated mechanism [13].

In our study, we try to elucidate why IPL systems (550–1200 nm) produce skin improvement, comparing different wavelengths. We use MiniSilk FT manufactured by Deka® to irradiate human cultured fibroblasts: IPL SA mode filter 800–1200 nm (frequency 10 Hz, emission time 15 s, fluence 60.1 J/cm^2) twice, IPL SA mode filter 550–1200 nm (frequency 10 Hz, emission time 15 s, fluence 60.1 J/cm^2) twice and IPL intense pulsed light mode (double pulse 5 ms+5 ms, delay 10 ms, fluence 13 J/cm^2) twice.

The purpose of this study is to asses and quantify that IPL applied to cultured fibroblasts has the ability to stimulate dermal matrix remodeling and to evaluate the effect of variations in wavelength and fluence on this stimulation. We examined and quantified gene expression of collagen I, III, metalloproteinase 1, hyaluronic acid, decorin, and versican in fibroblasts and synthesis of polysaccharides (PS) and proteoglycans (PG) of extracellular matrix by fibroblast: hyaluronic acid (HA), decorin and versican.

Material and methods

Cell culture

1BR3G human skin fibroblasts were derived from a normal fibroblast 1BR3 (ECACC catalogue no. 90011801) transformed with the plasmid pSV3gpt. 1BR3G (ECACC catalogue no. 90020507) were kindly provided by Dr. Josep Baullida.

Cells were grown in modified Eagle's medium (EMEM) with Earle's balanced salt solution supplemented with 1 % nonessential amino acids (NEAAs) (Sigma-Aldrich, St Louis, MO, USA), 2 mM glutamine, 56 IU/ml penicillin, 56 mg/l streptomycin, and 10 % fetal bovine serum (FBS) (BioWhittaker, Walkersville, MD). Cells were maintained in 100-mm tissue culture flask in a humidified chamber at 5 % CO_2 and 37 °C, and subcultured every 2 to 3 days by trypsin-EDTA (BioWhittaker, Walkersville, MD). For all subsequent experiments, 1BR3G cells were seeded in EMEM medium without phenol red (Lonza, Basel, Switzerland).

Irradiation

1BR3G human fibroblasts were seeded onto 60-mm culture plates in 4 mL of fresh culture medium without phenol red. After incubation for 1 day at 37 °C in 5 % CO_2, the monolayer of subconfluent cells was irradiated with MiniSilk FT manufactured by Deka®. Group 1 was irradiated with IPL SA mode filter 800–1200 nm (frequency 10 Hz, emission time 15 s, fluence 60.1 J/cm) twice. Group 2 was irradiated with IPL intense pulsed light mode filter 550–1200 nm (double pulse 5 ms+5 ms, delay 10 ms, fluence 13 J/cm^2) twice in one point at 2-cm distance. Nonoverlapping emissions were performed to homogeneously cover all the treatment petri area. Group 3 was irradiated with IPL SA mode filter 550–1200 nm (frequency 10 Hz, emission time 15 s, fluence 60.1 J/cm^2) twice. SA mode emits one unique long impulse during 15 s, constituted of short pulses with 10-Hz frequency. In SA mode, handpiece was moved in a slow motion creating and area about 5×5 cm which covered the culture plate, performing continuous, linear and flowing movements over the culture without holding the handpiece in one point at 2-cm distance. All irradiated groups showed a fibroblast increasing by flow cytometer (data not shown).

Quantification of mRNAs using real-time quantitative RT-PCR

Total RNA was isolated from cells (1×10^6) 48 h after irradiation with the RNeasy kit (Qiagen, Milan, Italy) as described by the manufacturer. First-strand complementary DNAs (cDNAs) were synthesized from 2 μg total RNA, using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), following the recommendations of the manufacturer. As a control for genomic contamination, the same reactions were performed in the absence of reverse transcriptase. All real-time PCR reactions were performed using the ABI Prism 7000 SDS (Applied Biosystems), and the amplifications were done using the SYBR Green PCR Master Mix (Applied Biosystems). FAM-labeled primers were distributed by Applied Biosystems and are as follows: hyaluronic acid (HYAL2; Hs01117343_g1), versican (VCAM;

Hs00171642_m1), decorin (DCN; Hs00754870_s1), type I collagen (COL1A1; Hs00164004_m1), type III collagen (COL3A1; Hs00943809_m1), and metalloproteinase 1 (MMP-1; Hs00899658_m1). Real-time PCR conditions were selected according to the universal conditions recommended by the manufacturer of the instrument. The experiments were carried out in duplicate for each data point. Basic analysis was performed using the SDS 1.9.1 software (Applied Biosystems). In addition, the expression of RNA 18S was used as housekeeping gene (calibrator) to standardize the relative expression of each experimental gene.

Picro-Sirius red staining

Picro-Sirius red (PSR) was purchased from Abcam (Cambridge, UK). In brief, cells cultured on glass coverslips were fixed in 4 % paraformaldehyde at room temperature, carefully washed twice with PBS. Thereafter, the nuclei were stained with hematoxylin, followed by a series of PBS washed. Subsequently, the slides were incubated in the staining solution PSR (0.1 %) at room temperature for 1 h. The staining solution was removed, and the cells were washed three times with 0.1 % acetic acid. For photography, cells on chamber slides were dehydrated and clarified by three changes of 100 % ethanol, 5 min each, followed by xylene, three changes, 10 min each, and coverslips were mounted with Permount (Electron Microscopy Sciences, Hatfield, PA, USA). The slides were examined by microscopy using an Axioskop 2 microscope using an oil immersion ×100 objective.

ELISA

The sensitive ELISA method was used to measure the fibroblast GAG protein expression. Confluent cultures of 1BR3G human were harvested with 0.25 % trypsin supplemented with 0.02 % EDTA. Supernatants were collected after centrifugation at 15,000 rpm for 15 min at 4 °C by refrigerated centrifuge (Eppendorf AG, Germany). Cell lysates were prepared using the freeze/thaw procedure.

Human HA ELISA kit (Cusabio Biotech Co., Ltd, China), with a detection limit of 0.156 ng/ml, was used to detect HA. Human versican ELISA kit (Cusabio Biotech Co., Ltd, China), with a minimum detection limit of 1.56 ng/ml, was used to detect versican. Human decorin ELISA kit (Cusabio Biotech. Co., Ltd, China), with a minimum detection limit of 1.56 ng/ml, was used to detect decorin. Briefly, samples were diluted with sample diluent and incubated in microtiter wells coated with antibodies of proteins. After incubation and washing, the biotinylated tracer antibody conjugated with streptavidin-peroxidase was added to the wells. Substrate tetramethylbenzidine (TMB) was added to the wells after a second incubation and washing, and then, the oxalic acid was added to stop the enzyme reaction. Absorbance was read on X-Fluor microplate spectrophotometer (Tecan Systems, Inc., USA) at a wavelength of 450 nm.

Statistical analysis

Statistical analyses were performed with the SPSS 15.0 software. The results were expressed as mean±SEM. Differences between the different groups were evaluated with Student's *t* test, and a p value <0.05was designated as statistically significant.

Results

Irradiation with IPL increases mRNA for collagen type I, type III, and MMP-1

The cultured fibroblasts were divided into three groups and treated with IPL radiation at doses previously described. The mRNA expression was measured after culturing irradiated fibroblast for 48 h.

The mRNA expression levels of procollagen type I (Fig. 1a) procollagen III (Fig. 1b), and MMP-1 (Fig. 1c) were increased ($p<0.05$) in all groups. The most significant changes of mRNA were found in group 3.

To confirm that mRNA increasing of collagen correspond with high intracellular collagen content, we chose Picrosirius red stain method. Picrosirius red in saturated picric acid solution specifically and consistently stain collagen I and III fibers. Analyses of Picrosirius red stained human fibroblast before (Fig. 2a) and after irradiation (Figs. 2b–d) revealed a significant increase in the amount of collagens I and III probably related to IPL treatment.

Irradiation with IPL increases HA synthesis in fibroblast cell line

After culturing for 48 h after irradiation, the levels of HA were firstly determined by real-time PCR. The results show that IPL irradiation significantly increased the expression of HA mRNA by 4–5-fold (Fig. 3a). The most significant results were obtained in group 2.

Moreover, to study and compare the effect of irradiation on the levels of this matrix protein, the cell lysates and the supernatants were assessed by ELISA. A significant increase of HA in lysed cells and supernatant

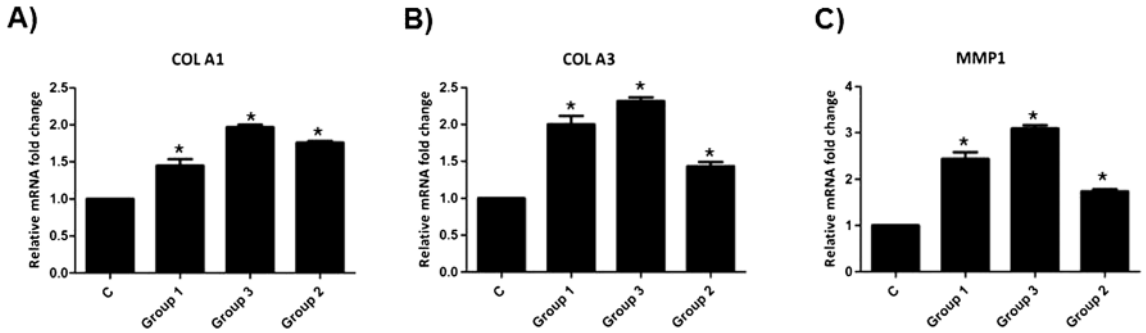

Fig. 1 Effects of IPL irradiation (mode SA) on collagen type I (COL A1) (**a**), collagen type III (COL A3) (**b**), and MMP-1 (**c**) expression. 1BR3G fibroblast cell line was cultured in 10 % CFS medium. Real-time RT-PCR analysis of the mRNA expression was carried out 48 h post-irradiation. The data represent the mean±SEM from triplicate determinations of at least three independent experiments for each case. *A significant difference compared with the control in all genes was found after IPL irradiation ($p<0.05$); 254×190 mm (96×96 DPI)

can be observed in all irradiated groups (Fig. 3b), with the highest increase in cell lysates. In this case, the most significant results were obtained in group 3.

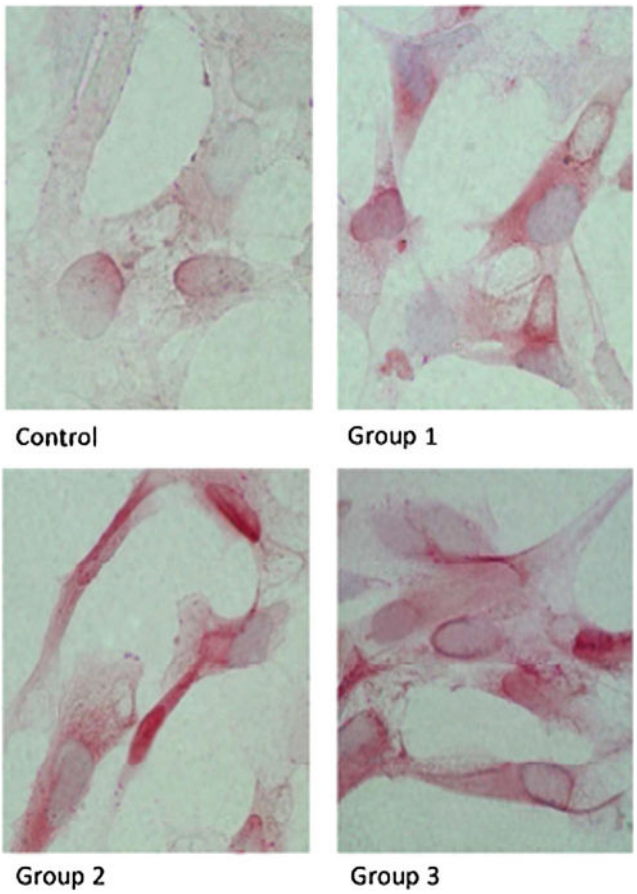

Fig. 2 1BR3G fibroblast picro-Sirius red staining after IPL irradiation. Human fibroblasts were cultured on glass coverslips and fixed and stained with PSR (0.1 %) as described in the "Material and methods" section. The nuclei were previously stained with hematoxylin. Control (**a**); group 1, IPL SA mode filter 800–1200 nm (**b**); group 2, IPL intensed pulse light mode filter 550–1200 nm (**c**); and group 3, IPL SA mode filter 550–1200 nm (**d**) images of showed the highest collagen content after fibroblast irradiation; 190×254 nm (72×72 DPI)

Irradiation with IPL increases versican synthesis in fibroblast cell line

After culturing for 48 h after irradiation, the levels of versican were firstly determined by real-time PCR. The results show that IPL irradiation significantly increased the expression of versican mRNA by 2–3-fold (Fig. 4a). The most significant results were obtained in group 3.

Moreover, to study and compare the effect of irradiation on the levels of versican, the cell lysates and the supernatants were assessed by ELISA. A significant increase of versican in lysed cells and supernatant can be observed in all irradiated groups (Fig. 4b), with the highest increase in supernatant and the most significant results in group 3.

Irradiation with IPL increases decorin secretion by fibroblast in supernatant

Decorin mRNA expression was quantified 48 h after IPL irradiation, with significant increase in groups 2 and 3 (Fig. 5a). Two days after treatment, the levels of decorin protein in cell supernatants and in lysed cells (Fig. 5b) were determined by ELISA. No significant increase in decorin levels can be observed in cell lysates. In supernatant, no changes are observed in group 1 but are increased in groups 2 and 3.

Discussion

IPL is a laser-like device that uses a flash lamp to produce a non-coherent pulsed light with variable pulse durations and intervals, mainly used in the treatment of certain skin diseases, including photoaging and telangiectasias, among others [14]. Most of the studies are limited to histological and clinical comparison [15]. Although some studies have been performed, mostly in vivo samples and focused on collagen production and MMPs regulation, specific molecular mechanism is still unknown [16, 17]. Our research is focused on human

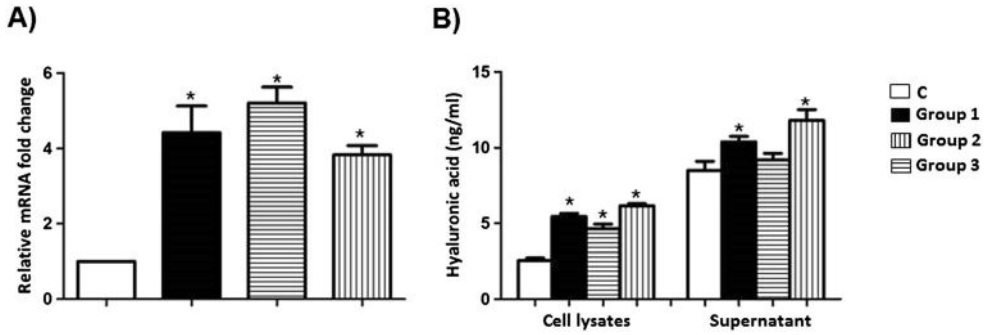

Fig. 3 Effects of IPL irradiation on hyaluronic acid in human skin fibroblast. 1BR3G fibroblasts were irradiated by IPL as described in the "Material and methods" section. Cells were harvested 48 h after irradiation, and HA mRNA expression was measured by real-time quantitative RT-PCR (**a**). Cell lysates and the supernatants were also collected, and HA secretion was also measured by ELISA kit (**b**). Triplicate wells were used, and the experiment was repeated three times (n=9). *p>0.05 as compared with the control fibroblast; 254×190 mm (96×96 DPI)

skin fibroblasts cell cultures to elucidate how IPL systems could produce dermal changes in gene expression and ECM proteins, including some poorly studied such as versican and decorine, and contribute to photorejuvenation, trying to compare if different parameters have influence over those potential changes.

Our study showed that IPL irradiation in all groups induces a significant skin fibroblast proliferation [13]. These results are in agreement with previous laser studies where an increase in the number of fibroblasts is associated with clinical improvement in wrinkle appearance. The precise mechanism responsible for increasing fibroblasts is unknown. Fibroblast cells exhibit specific splicing program independently of their tissue of origin [18]. Regarding to cell proliferation, some fibroblast growth factors (FGFs) have been described in tumoral processes [19] and in hypertrophic and keloid scars [20]. The binding of FGF and heparan sulfate proteoglican (HSPG) to the extracellular ligand domain of FGFR induces receptor dimerization, activation and autophosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor molecule. A variety of signaling proteins are phosphorylated in response to FGF stimulation leading to stimulation of intracellular signaling pathways that control cell proliferation, cell differentiation, cell migration, cell survival, and cell shape [21]. Our study was not focus on mechanism for increasing fibroblasts, so any tests have been performed searching that mechanism.

The ECM is a complex network of different structural proteins which includes proteins, matricellular proteins, proteoglycans, hyaluronan, and glycoproteins that interact and determine mechanical properties of the dermis. Age-related changes in dermal ECM are expected to be involved in age-related changes in the mechanical properties of skin [22]. The remodeling of skin collagen involves not only the proliferation of new collagen fibers but also the degradation of denatured collagens. MMPs are zinc-dependent endopeptidases that perform this degradative function, generally targeting the extracellular matrix [23]. They are capable of inducing ECM protein degradation and cleavage of cellular surface receptors. MMP-1 is called collagenase, and its main substrate are collagen types III, I, II, VII, and X [24]. Some authors [25] have reported a downregulation of mRNA expression of MMPs in a time-dependent fashion, suggesting that IPL irradiation could not degrade the protein components. They found major increases of mRNA during the first week after treatment. On the other hand, other authors have reported

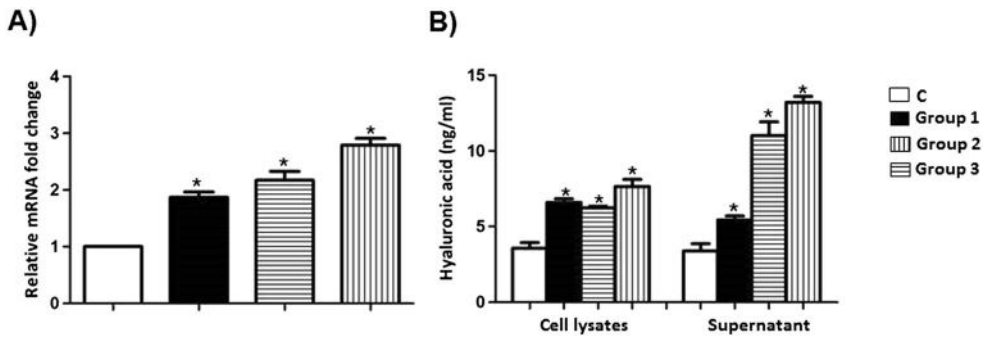

Fig. 4 Effects of IPL irradiation on versican in human skin fibroblast. Forty-eight hours after irradiation, the expression of versican mRNA was measured by real-time PCR (**a**). Intracellular and supernatant versican levels (ng/ml) were also analyzed by ELISA (**b**). The mean of data corresponding to three independent experiments in triplicate wells. Lines on top of the bars correspond to SEM. *Statistical evaluations comparing before and after IPL irradiation gave p<0.05 by paired t tests; 254×190 mm (96×96 DPI)

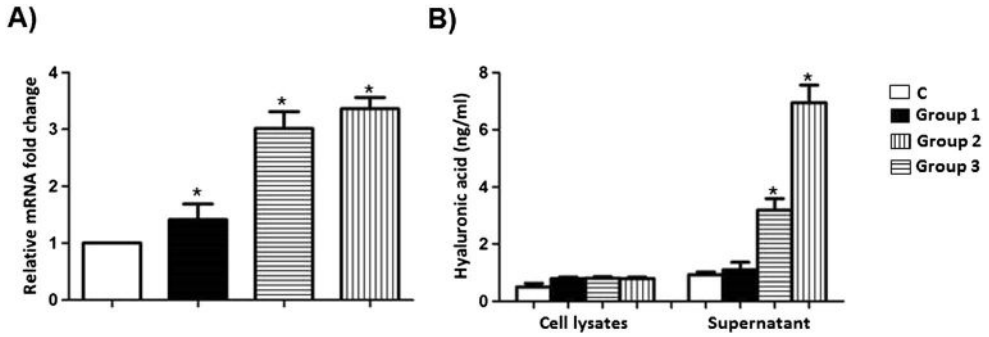

Fig. 5 Effects of IPL SA irradiation on decorin in human skin fibroblast. Two days after IPL SA irradiation, decorin mRNA level was detected by real-time PCR (**a**). Levels of decorin (ng/ml) in 1BR3G fibroblast were assessed by ELISA (b) using both cell lysates and supernatant. Data represent mean±SEM for three independent experiments performed in triplicate. *A significant difference compared to control was found ($p<0.05$); 254×190 mm (96×96 DPI)

upregulation of mRNA of MMP-1, MMP-3, and MMP-9 after Erbium:YAG laser treatment, most increased 1 day after treatment [26]. Our study showed that the expression of MMP-1 is increased 48 h after IPL irradiation [27]. Although it is well established that MMP expression was increased in damaged skin [28], some authors reported that the IPL management had no impact on MMP secretion levels in fibroblasts [29]. Some authors speculate that it could be an overlooked mechanism of skin rejuvenation, in which increased of MMP-1 will be implicated, contributing to the degradation of senescent collagens. Recent studies demonstrated that the significant differences in the expression of MMP (downregulation and upregulation) that may be related to the laser parameters such as wavelength and fluence [12, 30, 31], which is in accordance of our results. We suggest that these despaired results can be related to time of measurement and that MMPs downregulation and upregulation is time-dependent, observing an upregulation after treatment which converts into a downregulation the weeks after.

In our study, we can differentiate two tendencies according to the wavelength used. First, one is with HA and collagen, which show statistical significant differences between control and irradiated cells for all three groups. No statistical differences are observed in those groups treated with longer pulses or different wavelengths (550–1200 or 800–1200 nm).

Collagen is the predominant fibrous protein of the ECM; moreover, the dermis is mainly composed of type I and III collagens, which are responsible for providing major structural integrity to the dermis. Talwar et al. [23] reported that procollagen type I and III levels are decreased in photoaging and/or aged skin. Moreover, controlled thermal skin injury has been shown not only to increase of type I and III procollagen mRNA expression levels but also to change the structure and length of collagen [32]. Our study demonstrated that IPL treatment promotes the production of collagen I and III in all irradiated groups, which may have some association with IPL by direct stimulation and/or photothermolysis which is according with other authors [26, 33]. Most significant changes are observed in collagen III which is the first collagen deposited during wound repair and is gradually replaced by collagen I over time. In our study, collagen deposition was more increased in that group which received more total energy dose. This result is in accordance with Iyer [34] who suggests that maximizing fluence is more important in achieving optimal collagen deposition rather than maximizing the number of passes.

Although collagen is the major ECM molecule of the dermis, other molecular components are present contributing to the overall mechanical properties of skin. Among the noncollagenous components of the dermis, there are proteoglycans (PGs) and glycosaminoglycan conjugated proteins (GAGs), which are essential for maintaining mechanical strength of the skin. We carried out the detection of some of these ECM components: HA, versican, and decorin using sensitive method such as real-time RT-PCR and ELISA.

Our results showed a HA and versican increased expression after IPL treatment in mRNA and ELISA in cell lysates and supernatant. These observations are in agreement with previous data that demonstrate a significant higher proportion of versican and HA in fetal skin than adult skin [35]. These matrix components are thought to support the active cellular proliferation, migration, and differentiation events required for skin growth and development [36].

HA is the most abundant PG in dermis, synthetized in the plasma membrane of dermal fibroblast and secreted to the extracellular space with functions as ground substance to fill space in (ECM). In aged skin, HA is decreased in dermis and epidermis [37].

It is well known that fibroblasts with a collapsed morphology adopt a catabolic phenotype with downregulation production of type I collagen and upregulating production of MMPs [38]. Some authors have proposed mechanical stretching of fibroblast as cause of collagen production [39]. If we consider that fibroblasts morphology interacts to ECM and vice versa [40], more attention should be paid to these small molecules which stabilized collagen molecule: versican and decorine.

Second tendency is observed for PG: versican and decorin in which statistically significant changes are observed just when 550–1200-nm wavelength is used, independent on pulse duration or total fluence.

Versican is a large chondroitin sulfate proteoglycan found in the ECM distributed with elastic fibers in the human dermis [5] which controls numerous cellular events such as differentiation, migration, proliferation, and apoptosis. It can be found expressed not only in the dermis but also in the basal layer of the epidermis, hair follicles, and sweat glands [41, 42]. Versican also binds to HA via its N-terminal region and is the major HA-binding molecule and can impart viscous properties to cutaneous microfibrils [43]. Versican has also been found in highly proliferative tissues, such as tumors [20] and have differences in the total amount and disaccharide composition from tumors and normal dermis that correlates with the differences between fetal skin versican and adult skin versican [4].

Decorin, is an interstitial PG abundant in the dermal ECM [44]. It binds to multiple collagen types, mostly type I collagen and other proteins such as transforming growing factor beta [45] and modulates their activity [46]. In addition to this, evidence suggests that binding of decorin in the same region as the MMP-1 cleavage site inhibits collagen cleavage by MMP-1 [47, 48]. It is produced primarily by dermal fibroblast and is localized in dermal extracellular matrix [49]. Increase of decorin expression has been reported in a variety of fibrotic conditions including systemic sclerosis [50]. The role of decorin in aged skin is discussed. Some authors have reported differences in decorin distribution [51], increased levels after UVA radiation [51], decreased levels after solar ultraviolet irradiation [49, 52], or in aged skin in vivo [37]. In the present study, significant changes in decorin expression have been found in mRNA and ELISA determination in supernatant for groups 2 and 3. No significant changes have been observed in cell lysates, which could be explained because decorin is synthesized and immediately expulsed to ECM. This results contrast with previous data that demonstrate human skin decorin minimal age-related differences [4] and changes in decorin molecular size in aged skin where is significantly smaller than in young skin [53]. So, clearly, new experiments are necessary to elucidate the role of the decorin in dermis.

In our study, fibroblast cultures irradiated with SA mode, which emits one unique long impulse during 15 s, constituted of short pulses with 10-Hz frequency (groups 1 and 3) should show more changes as this pulse produces more tissue heating. Until now, the proposed theory for rejuvenation with IPL systems is based on water heating and fiber degradation and that more heating and protein denaturalization leads to better clinical results [9, 10]. With our study in vitro, we can hypothesize that biochemical mechanism can be, despite water heating, the direct stimuli of fibroblast by light which result in an increase of mRNA and synthesis of collagen and ECM proteins.

Conclusion

Although irradiation with IPL increases fibroblast proliferation, mRNA procollagen type I, III, and MPP-1 and some extracellular matrix proteins, such as HA and decorin, increase of ECM proteins shows two tendencies. Macromolecules are stimulated by IPL irradiation with independence of wavelength or type pulse while PG such as decorin and versican are depending on wavelength and stimulated with 550–1200 filter.

IPL treatment produces changes in vitro which can explain clinical results of aging improvement when is applied for antiaging treatment.

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